

## Antiphospholipid antibodies (aPL) in systemic lupus erythematosus. Are they specific tools for the diagnosis of aPL syndrome?

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### Abstract

**Objective** — Antiphospholipid antibody (aPL) specificity for aPL-related events was evaluated in systemic lupus erythematosus (SLE).

**Methods**—A study was carried out on 105 patients affected with SLE comparing the prevalence of lupus anticoagulant (LA) and IgG and IgM anticardiolipin antibodies (aCL) between patients with and without features of antiphospholipid syndrome (APS). Antiphospholipid antibody profile was subsequently evaluated in the aPL positive patients with and without aPL-related events, thus excluding the patients with complications of APS possibly due to factors other than aPL.

**Results**—LA showed a strong association with thrombosis and livedo reticularis, and IgG aCL with thrombosis and neurological disorders, while no clinical features were associated with IgM aCL. A considerable number of aPL positive patients with no aPL-related manifestations was also observed, suggesting the low specificity of aPL assays (54.4%). When studying the 60 aPL positive patients, LA was specific (91.3%) for the diagnosis of aPL-related thrombosis, whereas aCL were not specific, although IgG aCL mean levels were higher in patients with arterial thrombosis than in those without APS features.

**Conclusions**—LA but not aCL positivity is a specific tool for the diagnosis of thrombotic complications due to aPL in SLE.

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Antiphospholipid antibodies (aPL), such as lupus anticoagulant (LA) and anticardiolipin antibodies (aCL), are the immunological markers of the antiphospholipid syndrome (APS).<sup>1</sup> In this condition aPL are found in association with venous and/or arterial thrombosis at every site and/or recurrent pregnancy loss.<sup>1</sup> Moreover, other manifestations including livedo reticularis, various neurological disorders, thrombocytopenia and positive direct Coomb's test can occur.<sup>1</sup> However, aPL may also be found in a large spectrum of other autoimmune and non-

autoimmune diseases<sup>2,3</sup> and characteristically in systemic lupus erythematosus (SLE).<sup>4,5</sup> The detection of aPL has been proposed as a predictive and specific tool for the diagnosis of APS in SLE.<sup>5</sup> The clinical significance of aPL in SLE patients, however, has not yet been well established,<sup>6,7</sup> partly because of a limited standardisation method applied in aPL detection.<sup>4,8</sup>

First, we studied LA activity and IgG and IgM aCL prevalences in SLE patients with and without clinical features of APS, to discover whether differences in aPL prevalence and characteristics could account for different clinical pictures. We then evaluated aPL profile in aPL positive SLE patients with and without aPL-related manifestations, thus excluding patients with complications of APS due to factors other than aPL. Such evaluation enabled us to a better definition of LA and aCL specificity for the occurrence of aPL-related events within SLE.

### Patients and methods

Serum and plasma samples of 105 patients affected with SLE (ARA criteria) were tested for aPL. There were 97 women and eight men, mean age 35.7 years, range 14-79 years. At the time of blood sampling, clinical history, physical examination and routine blood analysis were obtained from all patients to evaluate the presence of features related to aPL, particularly venous and/or arterial thrombosis, recurrent fetal loss, thrombocytopenia (platelet count below 100 000/ $\mu$ l in two different occasions), neurological disorders and livedo reticularis. We excluded eight patients treated with high doses of steroids (prednisone  $\geq$ 20 mg/daily) or immunosuppressants due to reports on the suppressive effects of this therapy on LA activity and, to a lesser extent, on aCL.<sup>5,9,10</sup> None of the patients were receiving thrombosis-inducing drugs such as oral contraceptives or being treated with heparin.

LA was determined by the diluted Russell viper venom time according to Thiagarajan *et al.*<sup>11</sup> Serum IgG and IgM aCL were measured by an enzyme-linked immunosorbent assay (ELISA) as described by Gharavi *et al.*<sup>12</sup> Results were expressed as GPL and MPL obtained from the standard curve of each plate. The mean value +4 standard deviations of 100

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Table 1 Frequency (percentage) of aPL, LA, IgG and IgM aCL in 105 SLE patients in relation to aPL syndrome manifestations.

n pts	aPL-related manifestations		Thrombosis		Fetal loss §		Thrombocytopenia		CNS disorders		Livedo reticularis	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
	57	48	87	18	45	2	89	16	85	20	96	9
aPL	45.6 <sup>^^</sup>	70.8	52.9	77.7	55.5	0.0	55.0	68.7	51.8*	80.0	53.1 <sup>^^</sup>	100.0
LA	3.5	14.6	4.6 <sup>ç</sup>	27.8	8.9	0.0	10.1	0.0	7.0	15.0	6.2**	33.3
IgG aCL	40.3 <sup>^</sup>	64.6	46.0 <sup>^</sup>	77.8	46.7	0.0	49.4	62.5	45.8**	75.0	48.9	77.8
IgM aCL	15.8	37.5	25.3	27.8	26.7	0.0	23.6	37.5	23.5	35.0	23.0	55.5

§ Out of 47 female patients who experienced pregnancy. \*p < 0.05; \*\*p < 0.04; ^p < 0.03; ^^p < 0.02; ç p < 0.007.

Table 2 Prevalence (percentage) of LA, IgG and IgM aCL in 60 aPL positive patients with SLE with and without clinical manifestations of aPL syndrome.

n pts	Thrombosis		Venous thrombosis		Arterial thrombosis		Thrombocytopenia		CNS disorders		Livedo reticularis	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
	46	14	49	11	55	5	49	11	44	16	51	9
LA	8.7*	35.7	12.3	27.3	11.0**	60.0	18.4	0.0	13.6	18.7	11.8	33.3
IgG aCL	87.0	100.0	87.7	100.0	89.1	100.0	89.8	90.9	88.6	93.7	92.1	77.8
IgM aCL	48.0	37.5	47.0	36.4	45.4	40.0	43.0	54.5	45.4	43.7	43.1	55.5

\*p = 0.04; \*\*p = 0.022.

healthy subjects (13 GPL and 15 MPL) was considered as cut-off for both IgG and IgM aCL levels.

Association of aPL and clinical manifestations was tested by Chi-square analysis of contingency tables. Mean (SD) differences of aCL levels, between the groups were determined by *t* test of independent samples.

**Results**

Raised levels of aPL were detected in the sera of 60 of 105 patients with SLE (57.1%): IgG aCL in 54 (51.4%), IgM aCL in 27 (25.7%), both IgG and IgM aCL in 21 (20.0%). LA was found in nine patients (8.6%), always associated with aCL positivity.

Eighteen patients (17.1%) had a history of at least one thrombotic event or episodes of vascular occlusions at the time of aPL detection: 12 had venous occlusions at lower and upper limbs, and abdominal veins in one case, 3 had arterial thrombosis (myocardial infarction, retinal and renal artery occlusions, respectively), and 3 had both types of

complications. Of 47 female patients who experienced pregnancy, only two (4.2%) had had two episodes each of fetal loss, eight (17%) had pre-term delivery and two (4%) pre-eclampsia. Sixteen patients (15.2%) had thrombocytopenia; 20 (19.0%) suffered from various neuropsychiatric disorders such as transient ischaemic attacks (n = 1), chorea (n = 1), epilepsy (n = 8), amaurosis fugax (n = 2), migraine (n = 1), psychosis (n = 7), and acute organic syndrome (n = 2); two patients had more than one neurological feature. Nine (98.6%) had livedo reticularis. Sixteen patients (15.2%) presented more than one manifestation. Fifty seven patients (54.3%) did not show any of the disorders mentioned.

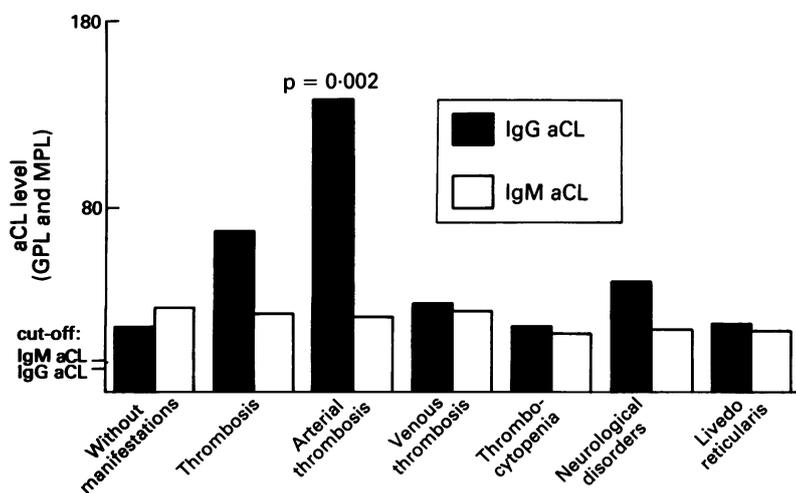
Frequency of aPL, LA, IgG and IgM aCL in SLE patients with and without aPL-related manifestations is reported in table 1. No difference in frequencies of pre-term delivery and pre-eclampsia between aPL positive and negative patients was found.

Frequency of LA, IgG and IgM aCL and mean IgG and IgM aCL levels in the 60 aPL positive patients with SLE, with and without clinical manifestations of APS, are shown in table 2 and the figure.

**Discussion**

The frequency of aCL in our patients was similar to that reported by other authors.<sup>6 7 9 13 14</sup> The frequency of LA was lower than the average reported by Love *et al*,<sup>4</sup> but similar to that found by some authors.<sup>4</sup> Both aCL and LA detection are influenced by differences in sensitivity and specificity of methods applied,<sup>8 15</sup> in the definition of the positive/negative cut off points<sup>8</sup> and in patient selection criteria.<sup>4 5</sup>

Our data (table 1) show that thrombosis was strongly associated with LA activity and, to a lesser extent, with IgG aCL positivity. Neurological symptoms were related to IgG aCL and livedo reticularis to LA. Recurrent



Mean levels of IgG and IgM aCL in aPL positive patients with and without aPL syndrome manifestations.

fetal loss and thrombocytopenia did not correlate with LA or aCL positivity. Recurrent fetal loss was an uncommon feature in our patients probably because aspirin was used at low doses in risk pregnancies. Previously, no direct relationship with aPL, both in SLE and non-SLE patients, has been reported in thrombocytopenia.<sup>4</sup> No selected manifestations were significantly correlated with IgM aCL; this agrees with a previous report.<sup>13</sup> Conversely, there was a considerable number of aPL positive patients with none of the selected manifestations (table 1): two were both positive for LA and aCL. Similar findings have been reported<sup>5 15</sup> but, as yet, have not been clarified. Such observations could suggest that methods for aPL detection are sensitive but not specific for identifying the subset of patients with SLE at risk for aPL-related complications.

Thus we investigated the clinical significance of LA and aCL characteristics (isotype and level) within the group of aPL positive SLE patients (table 2, figure). Our study group was better defined by excluding patients who did not have the criteria for APS, that is, patients with selected manifestations of APS not related to aPL positivity. Many other factors, however, may contribute to thrombotic diathesis in SLE.<sup>8</sup>

LA still appeared strongly correlated to thrombotic events, particularly arterial ones, but its association with livedo reticularis was not confirmed. Conversely, no difference in the prevalence of both IgG and IgM aCL between aPL positive patients with or without complications was observed, thus suggesting the low specificity of aCL detected by ELISA as a risk factor for the occurrence of aPL-related events in SLE.<sup>6 13 15</sup> Moreover, we investigated whether or not the definition of aCL level could have some clinical value (figure). We found that the mean level of IgG aCL was significantly higher in patients with arterial thrombosis than in those without any complication ( $p = 0.002$ ), whereas, no significant difference was found in the IgM aCL mean level.

In conclusion, LA is the most specific parameter for the diagnosis of thrombotic complications due to aPL in patients with SLE; both IgG and IgM aCL are not specific for aPL-related events. In addition, the measurement of IgG aCL level seems to be a valuable requirement before attributing clinical value to aCL positivity.

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